PPM1H is differentially expressed in brain metastases in breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Breast cancer patients with metastasis to the brain are faced with limited treatment options¹. To facilitate enhanced understanding of the transcriptional makeup of brain metastasis in human breast cancer and to support discovery of novel therapeutic targets, we mined published microarray datasets^{2,3} to identify genes whose expression was most markedly different when comparing brain metastases to primary tumors from patients with breast cancer and to the normal breast. We identified the gene encoding the protein phosphatase PPM1H as among the genes whose expression was most significantly different in metastases to the brain as compared to primary tumors of the breast. Increased expression of PPM1H in brain metastases in patients with breast cancer may be important for any number of steps involved in metastasis, including colonization of the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, systems biology of breast cancer, PPM1H.

Brain metastasis is a clinical problem in patients with breast cancer. One study described existence of central nervous system metastasis in 309 out of 1044 patients, with 193 metastases of these patients possessing metastasis specifically to the brain⁴. Overall median survival was reported as 7.4 months in breast cancer patients with central nervous system metastases⁵. Treatment options for patients with central nervous system metastases include whole-brain radiotherapy⁶, surgery⁷ and stereotactic surgery⁸, and treatment options for patients with metastatic breast cancer in general include capecitabine⁹, lapatinib and capecitabine⁹, neratinib and capecitabine¹⁰, trastuzumab¹¹, pertuzumab¹¹, and trastuzumab emtansine¹². To identify understand the molecular nature of brain metastasis in patients with breast cancer, and to support identification of potential therapeutic targets, we performed differential gene expression profiling using published microarray datasets^{2,3}. We identified PPM1H as among the genes whose expression was most significantly different transcriptome-wide when comparing brain metastases to primary tumor and normal breast from patients with breast cancer. PPM1H may be important to the biology of metastasis to the brain in patients with breast cancer whose primary tumors spreads to the central nervous system.

Methods

We used datasets GSE43837² and GSE52604³ for this global differential gene expression analysis in conjunction with GEO2R. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether PPM1H gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool¹³ for correlation of PPM1H mRNA expression levels with overall survival (OS) in $n=626$ breast cancer patients.

Results

PPM1H is differentially expressed in brain metastases in human breast cancer.

Comparison of global gene expression profiles of 19 primary tumors of the breast to 19 brain metastases² revealed that PPM1H was among the genes whose expression was most significantly different transcriptome-wide between primary tumors in patients with breast cancer and brain metastases (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and primary breast tumors, PPM1H ranked 2 out of 61359 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of PPM1H was statistically significant (Table 1; $p=0.00000698$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher

1 levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus,
2 we asked whether we could validate differential expression of PPM1H when comparing brain
3 metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the
4 breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases³
5 revealed that PPM1H was among the genes whose expression was most significantly different
6 transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2).
7 When sorting each of the genes expressed in brain metastases based on significance of difference in
8 expression between brain metastases and normal breast tissues, PPM1H ranked 2909 out of 41093 total
9 transcripts (Table 2), equating to 92.9% differential expression. Differential expression of PPM1H was
10 statistically significant in this dataset as well (Table 2; $p=2.06E-05$).

11 **PPM1H is expressed at significantly higher levels in brain metastases in human breast cancer.**

12 We obtained exactly mRNA expression levels for PPM1H in brain metastases and primary breast
13 tumors to evaluate magnitude and direction of change in PPM1H expression in metastasis to the brain in
14 patients with breast cancer. PPM1H expression was higher in metastases to the brain when compared to
15 primary tumors of the breast (Figure 1). Increased expression of PPM1H in brain metastases was
16 statistically significant (Figure 1: $p=0.0022$). We calculated a fold change in PPM1H expression of $4.44 \pm$
17 4.14 in brain metastases when compared to primary tumors of the breast.

18 **PPM1H expression in primary tumors of the breast is associated with overall survival in patients with breast cancer.**

19 A previous study reported that breast cancer patients with low tumor expression of PPM1H
20 trended towards worse survival¹⁴. Low expression of PPM1H was reported as a negative prognostic
21 indicator¹⁴. We performed Kaplan-Meier survival analysis to assess this issue, evaluating a correlation
22 between PPM1H mRNA primary tumor expression levels and overall survival in 626 breast cancer
23 patients. We found a statistically significant correlation between PPM1H expression and overall survival
24 (OS) in patients with breast cancer (Figure 2; log rank p -value: 0.037 for overall survival, hazard ratio:
25 1.4 (1.02 - 1.92) (Fig. 2)). We found that low PPM1H mRNA levels in primary tumors of the breast were
26 a positive and not negative prognostic indicator. Median OS was 135.84 months for patients whose
27 tumors expressed low levels of PPM1H while median OS was 69.73 months for patients whose tumors
28 expressed high levels of PPM1H (Table 3).

Thus, we identified PPM1H as among the genes whose expression was most significantly
different when comparing global gene expression between brain metastases and primary tumors of the
breast in patients with breast cancer; PPM1H expression was significantly higher in brain metastases as
compared to primary tumors of the breast. Significantly, we found that PPM1H expression in primary
tumors of the breast served as a prognostic indicator of overall survival in breast cancer, with patients
with low expression of PPM1H surviving significantly longer than those with high primary tumor
expression of PPM1H.

29 **Discussion**

We undertook a systems-level, unbiased approach to describing the most significant
transcriptional features of brain metastases in patients with breast cancer using published microarray
datasets^{2,3}, both to further describe the molecular characteristics of brain metastases¹⁵ and to support
identification of novel therapeutic targets in a clinical problem area. We identified the PPM1H receptor

1 as among the genes whose expression was most significantly different in brain metastases of humans with
2 breast cancer as compared to primary tumors of the breast. PPM1H was expressed at significantly higher
3 levels at the messenger RNA level in brain metastases as compared to primary tumors of the breast and as
4 compared to normal breast tissue.

5 The literature provides a description of PPM1H as a molecule involved in resistance to
6 trastuzumab¹⁴, with depletion of PPM1H conferring trastuzumab resistance to via reduced p27 levels, as
7 PPM1H could dephosphorylate p27 at threonine residue 187 which served as a signal for proteasomal
8 degradation; low tumor expression of PPM1H was reported as trending towards worse survival for
9 patients with breast cancer. However, our analyses are in stark contrast with these reports, as we find that
10 low primary tumor expression of PPM1H is significantly correlated with superior overall survival in
11 patients with breast cancer, with a difference of overall survival greater than 5 years based on PPM1H
12 expression. Increased expression of PPM1H in the metastatic brain tissues of patients with breast cancer
13 should be validated; PPM1H may serve a critical therapeutic target in an area with limited treatment
14 options: breast cancer patients with metastases to the brain. We provided evidence here that a druggable
15 protein phosphatase is among the genes most differentially expressed in the brain metastases of patients
16 with breast cancer, expressed at significantly higher levels in brain metastases as compared to primary
17 tumors of the breast, and that lower expression of this protein phosphatase in primary tumors of the breast
18 was associated with superior overall survival in patients with breast cancer.
19
20
21
22
23
24
25
26
27
28

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. McMullin, R.P., Wittner, B.S., Yang, C., Denton-Schneider, B.R., Hicks, D., Singavarapu, R., Moulis, S., Lee, J., Akbari, M.R., Narod, S.A. and Aldape, K.D., 2014. A BRCA1 deficient-like signature is enriched in breast cancer brain metastases and predicts DNA damage-induced poly (ADP-ribose) polymerase inhibitor sensitivity. *Breast Cancer Research*, 16(2), pp.1-10.
3. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *PloS one*, 9(1), p.e85448.
4. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
5. Witzel, I., Laakmann, E., Weide, R., Neunhöffer, T., Park-Simon, T.J., Schmidt, M., PPM1Hching, P.A., Hesse, T., Polasik, A., Mohrmann, S. and Würschmidt, F., 2018. Treatment and outcomes of patients in the Brain Metastases in Breast Cancer Network Registry. *European Journal of Cancer*, 102, pp.1-9.
6. Niwińska, A., Murawska, M. and Pogoda, K., 2010. Breast cancer subtypes and response to systemic treatment after whole-brain radiotherapy in patients with brain metastases. *Cancer*, 116(18), pp. 4238-4247.
7. Patchell, R.A., Tibbs, P.A., Walsh, J.W., Dempsey, R.J., Maruyama, Y., Kryscio, R.J., Markesbery, W.R., Macdonald, J.S. and Young, B., 1990. A randomized trial of surgery in the treatment of single metastases to the brain. *New England Journal of Medicine*, 322(8), pp.494-500.
8. Kondziolka, D., Kano, H., Harrison, G.L., Yang, H.C., Liew, D.N., Niranjan, A., Brufsky, A.M., Flickinger, J.C. and Lunsford, L.D., 2011. Stereotactic radiosurgery as primary and salvage treatment for brain metastases from breast cancer. *Journal of neurosurgery*, 114(3), pp.792-800.
9. Geyer, C.E., Forster, J., Lindquist, D., Chan, S., Romieu, C.G., Pienkowski, T., Jagiello-Grusfeld, A., Crown, J., Chan, A., Kaufman, B. and Skarlos, D., 2006. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *New England journal of medicine*, 355(26), pp.2733-2743.

10. Freedman, R.A., Gelman, R.S., Anders, C.K., Melisko, M.E., Parsons, H.A., Cropp, A.M., Silvestri, K., Cotter, C.M., Componeschi, K.P., Marte, J.M. and Connolly, R.M., 2019. TBCRC 022: a phase II trial of neratinib and capecitabine for patients with human epidermal growth factor receptor 2–positive breast cancer and brain metastases. *Journal of Clinical Oncology*, 37(13), p.1081.
11. Swain, S.M., Baselga, J., Kim, S.B., Ro, J., Semiglazov, V., Campone, M., Ciruelos, E., Ferrero, J.M., Schneeweiss, A., Heeson, S. and Clark, E., 2015. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *New England Journal of Medicine*, 372(8), pp.724-734.
12. Verma, S., Miles, D., Gianni, L., Krop, I.E., Welslau, M., Baselga, J., Pegram, M., Oh, D.Y., Diéras, V., Guardino, E. and Fang, L., 2012. Trastuzumab emtansine for HER2-positive advanced breast cancer. *New England Journal of Medicine*, 367(19), pp.1783-1791.
13. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.
14. Lee-Hoeflich, S.T., Pham, T.Q., Dowbenko, D., Munroe, X., Lee, J., Li, L., Zhou, W., Haverty, P.M., Pujara, K., Stinson, J. and Chan, S.M., 2011. PPM1H is a p27 phosphatase implicated in trastuzumab resistance. *Cancer discovery*, 1(4), pp.326-337.
15. Bos, P.D., Zhang, X.H.F., Nadal, C., Shu, W., Gomis, R.R., Nguyen, D.X., Minn, A.J., van de Vijver, M.J., Gerald, W.L., Foekens, J.A. and Massagué, J., 2009. Genes that mediate breast cancer metastasis to the brain. *Nature*, 459(7249), pp.1005-1009.

Rank	ID	p-value	t	B	FC	Gene	Gene name
2	Hs.21894.0.S1_3p_at	0.00000698	5.1156268	3.12569	4.44 ± 4.14	PPM1H	protein phosphatase, Mg2+/Mn2+ dependent 1H

Table 1: PPM1H is differentially expressed in the brain metastases of patients with breast cancer.

Rank of differential expression relative to all transcripts measured by microarray, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing brain metastases to primary tumors of the breast, the fold change of PPM1H expression in brain metastases when compared to primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2909	A_23_P391443	2.06E-05	4.7342259	2.469745	PPM1H	protein phosphatase, Mg2+/Mn2+ dependent 1H

Table 2: PPM1H is differentially expressed in the brain metastases of patients with breast cancer.

Rank of differential expression relative to all transcripts measured by microarray, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing brain metastases to normal breast tissue, gene and gene name are listed in this chart.

Figure 1: PPM1H is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

PPM1H mRNA expression levels in primary tumors of the breast (left) and in metastases to the brain (right) are depicted here with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in PPM1H mRNA expression between primary tumors of the breast and brain metastases, a *p*-value, listed above. Scale of the y-axis is presented in natural logarithm (ln).

Figure 2: PPM1H expression correlates with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=626$ total patients stratified into two groups, based on low or high expression of PPM1H in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on PPM1H expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
135.84	69.73

Table 3: Median overall survival is greater in breast cancer patients with low primary tumor expression of PPM1H.

The overall survival of $n=626$ breast cancer patients based on stratification into low or high tumor expression of PPM1H is listed in this chart.